MILK PRODUCTION ON A PROTEIN-FREE AND PROTEIN-POOR FEED *

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The human body, apart from water, is mainly composed of proteins. Of the dry substance of a fat-free body about 78 % is protein and about 18 % minerals. All enzymes are specific proteins which rule and direct the thousand or more various chemical reactions of metabolism. The proportion of carbohydrates in the fat-free dry substance of the body is small, about 4 %. On the contrary, in the food of man carbohydrates occupy quantitatively a major position if the use of too much fat does not take the place of a large part of them. Roughly speaking the diet of man contains 80 % carbohydrates + fat, and 10–15 % protein. The composition of the food and that of the body are thus quite different. Proteins form the machinery of the body, the propelling power of which is energy nutrition: carbohydrates and fat. Because the machinery of the body is continuously decomposed, proteins must regularly be included in the food. The growing organism in particular needs plenty of protein, and therefore any deficiency of protein can cause irreparable damage in the organism of children.

As the proteins received from the food are decomposed in the alimentary canal into amino acids which are then absorbed into the blood, the organism always forms its own proteins from amino acids. Therefore the protein problem is in fact a problem of amino acids. The eight essential amino acids, which man's organism is not able to synthesize and which must therefore be received from the food, decide the protein state of man.

Well-nourished populations receive a considerable proportion or even the major part of their proteins directly from animal products. All the necessary amino acids are found in them, and they are often in suitable proportions.

The cow has a key role as a producer of protein because it gives both milk and meat, the proteins of which have a high biological value. Milk has a special value in the nutrition of children and also adults because of its many-sided and

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valuable composition. If the vegetable diet consisting mostly of cereals, which is normal for most of the worlds's population, could be supplemented by half a litre of milk per person per day, malnutrition would practically disappear.

Since great losses occur when plant protein is changed into animal protein, it has been questioned whether there will be, in the future, any possibility of production of animal protein in a more and more overcrowded world. In milk production the daily feed has to contain roughly 60 grams of digestible crude protein for each kilogram of milk produced. This is nearly twice as much protein as there is in 1 kilogram of milk. Because utilization of nutrients in ruminants is very different from the utilization in other mammals, the fermentation processes in the rumen caused by microbial flora being of decisive importance, the old idea is logical that protein to some extent can be formed in the rumen from simple nitrogen compounds. Large numbers of feeding experiments have been made in different countries to find out how much of the protein can be successfully replaced by urea, which is readily decomposed to ammonia in the rumen. It has been possible to recommend only small amounts of urea in practice.

Experiments (mostly in the USA) with growing lambs, goats, and steers on protein-free feed, having urea as the only significant source of nitrogen, have shown that optimum growth was not obtained in spite of the relatively small amounts of protein which are needed for the growth of young ruminants. It is thus understandable that milk production, for which many times more protein is needed, has not been investigated with a protein-free feed.

However, many problems regarding the formation of different milk components actually require the study of milk production on a purified feed, with urea and ammonium salts as the sole sources of nitrogen. Such a study is of course also fundamental from a practical point of view.

Our studies on this type of feeding were started in connection with a research project concerning the origin of the flavour substances of milk. The question under study was to what extent the normal flavour substances are due to synthesis within the cow's body and to what extent they are derived from the feed. It was thus important to prepare a test feed which was as simple and as pure as possible.

To obtain information on the use of ammonium nitrogen for the synthesis of milk proteins a preliminary feeding experiment was performed in the spring of 1958, in which a cow on normal feed was given one dose of ammonium sulphate labelled with ^{15}N. The degree of labelling of the amino acids, separated by fractionation after hydrolysis of the milk proteins, was determined. It was found that in the milk obtained 15 hours after the feeding of ^{15}N-ammonium sulphate all the protein amino acids studied were labelled, but that the degree

of labelling of the various amino acids differed. In later experiments the labelling of all the amino acids in milk protein could be demonstrated 3 hours after the ^{15}N-feeding. The essential amino acids were labelled more slowly than most of the non-essential ones; this was natural on the assumption that the essential acids are synthesized only in the rumen by micro-organisms, whereas the non-essential are also formed in other parts of the body, particularly in the liver, from ammonia. The strongest labelling of glutamic acid is thus understandable.

The slower labelling of some essential amino acids in milk protein suggested that their synthesis may form a bottleneck in protein synthesis. Thus the possibility of developing in the rumen, by adaptation to a test feed, a microbial flora which could synthesize protein from ammonia more effectively than could the rumen micro-organisms of cows on normal feed and thus make milk production possible seemed to merit experimental study.

The experiments on protein-free feed with urea and ammonium nitrogen as the sole sources of nitrogen were started with one cow in the autumn of 1961 and with another at the beginning of 1962. A very slow adaptation procedure was used. When one of the test cows (Eiru) was given a dose of ^{15}N-labelled urea, after having been on the test feed for 6 months, and the labelling of the amino acids of milk protein was quantitatively determined at different times after the administration of ^{15}N, the labelling of many essential amino acids was found to be strongly increased relative to values obtained in experiments with a cow on normal feed. Another experiment, in which ^{15}N-ammonium sulphate was given to the same test cow after it had been on test feed for 25 months, gave similar results. The curves in Fig. 1 illustrate the effect of adaptation to the test feed on the labelling of the essential amino acids of milk protein. The composition of the test feed used in our experiments is given in Table 1. The amount of nitrogen-containing impurities in the test feed is so small that the urea and ammonium nitrogen account for 99.5 per cent of the total nitrogen.

Different test cows were given briquettes, cellulose-rich paste, and cellulose strips in different proportions according to appetite. Thus the feed of one cow differed from the feed of another to some extent. The test feed of different cows contained potato starch, cellulose, and sucrose in the following proportions: potato starch 46 to 62 per cent of the total carbohydrates; cellulose 24 to 38 per cent; sucrose 15 to 23 per cent.

The test cows were fed twice a day. When the daily rations were high they ate the rations gradually over a period of some hours and in this way regulated the intake of urea. There was thus no need to divide the feed repeatedly into small portions. In contrast, the dry or low-milking cows consumed their smaller rations in a very short time.

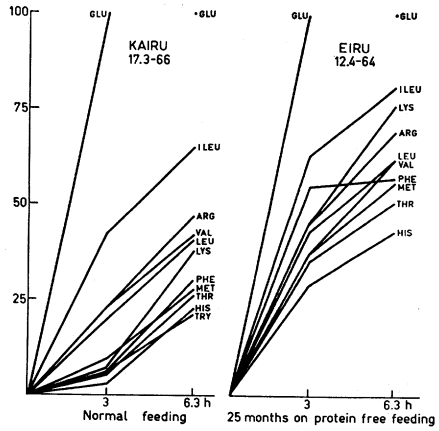


Fig. 1. The labelling of the essential amino acids of the proteins of milk with ^{15}N 3 and 6.3 hours after the cows had been given a single dose of ^{15}N-urea. The results are given as the percentage of the labelling of glutamic acid, since this amino acid is always labelled most rapidly. On the left are the results of a cow on normal feed, on the right those of a cow which has been on test feed for 25 months. Determinations by M. Kreula and T. Moisio.

The nitrogen content of the feed was raised considerably during the experiment. The largest amount of urea (ammonium nitrogen included) used during the first 2 years was only a little more than 400 grams per day during the period of highest milk production. We began to raise the amount of nitrogen in the feed in autumn 1963; since autumn 1965 the cows, weighing about 450 kilograms each, have received as much as 650 grams and in occasional cases even nearly 700 grams urea per day depending on the intake of carbohydrates. Ammonia poisoning has never occurred.

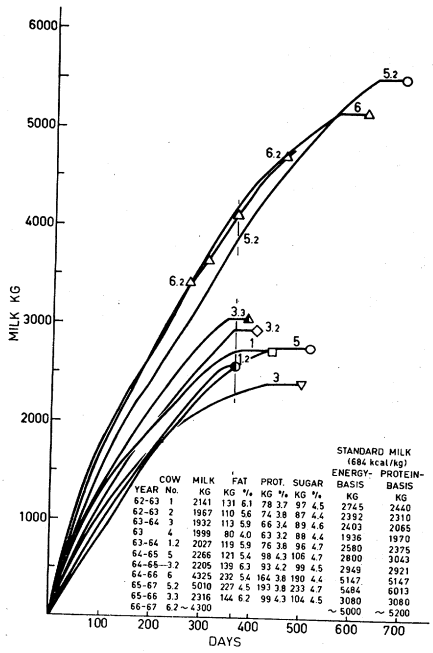
The digestibility of the urea and the nitrogen balance were determined several times. Raising the urea content in the feed enhanced the digestion coefficient for urea nitrogen from 63 ± 0.97 to 70 ± 1.2 when the nitrogen consumed (in grams of nitrogen per kilogram of organic feed) was raised from about 16-18 to 23-25. The increase was statistically highly significant. The nitrogen balance was + 12.2 on average.

The raising of the urea content of the diet removed a probable symptom of nitrogen deficiency which was observed when the feed with lower urea content was used: the continuous thinning or loss of the hairy coat of the fore part of the cows' legs, especially that of the hind legs, about 2 months after calving, and the rapid regrowth when the daily milk yield had decreased to about 7 kilograms. This phenomenon disappeared when more urea was fed.

Table 1. Components of the feed of the test cows since 1965.

1. Composition of the briquettes, about 9 g each:	
α-cellulose powder	9.9%
Starch	52.9%
Sucrose	23.1%
Mineral salt mixture	8.9%
Urea + amm. salts	5.2%
Total	100.0%
Water content	15.0%
2. Composition of the wet cellulose paste:	
α-cellulose powder	57.3%
Starch	16.4%
Sucrose	12.2%
Mineral salt mixture	8.8%
Urea	5.3%
Total	100.0%
Mixed with water, water content	75.0%
During a high milk production a sufficient amount of urea is not found in the basic feed. If needed, more urea could be mixed with the paste or the sugarfree briquette powder, which was occasionally used.	
3. Cellulose strips + 4% urea. Most test cows do not eat them.	
4. Vegetable oils 100–130 g/cow/day	
5. Vitamins A, D_2 and D_3, and vitamin E for trial	
6. Mineral salt mixture	

Altogether six cows have been given the experimental feed (Fig. 2). The first four test cows (all Ayrshire breed) were chosen as representatives of cows with a relatively low milk yield (2500 to 3500 kilograms of milk and 104 to 154 kilograms of fat per year) on normal feed because the protein requirement of such cows can more easily be satisfied. Only Cow No. 6 (Metta, 9 years old) had produced on normal feed during 6 lactation periods as much as 4000 to 6000 kilograms of milk and 167 to 244 kilograms of fat per year. The feed adaptation of Cow No. 5 (Jairu) was started when she was a heifer, 2 months before her first calving. Figure 2 shows the annual yield of milk, milk fat, milk protein, and milk sugar for each of the test cows on the experimental feed for various lactation periods. It also shows the milk yields per year and the length of lactation periods. For comparison the milk yields are presented as standard milk, which corresponds to 684 Kcal/kg milk, *e.g.* milk with 4.0 % fat, 3.2 %



YEAR	COW No.	MILK KG	FAT KG	FAT %	PROT. KG	PROT. %	SUGAR KG	SUGAR %	STANDARD MILK (684 kcal/kg) ENERGY-BASIS KG	STANDARD MILK (684 kcal/kg) PROTEIN-BASIS KG
62-63	1	2141	131	6.1	78	3.7	97	4.5	2745	2440
62-63	2	1967	110	5.6	74	3.8	87	4.4	2392	2310
63-64	3	1932	113	5.9	66	3.4	89	4.6	2403	2065
63	4	1999	80	4.0	63	3.2	88	4.4	1936	1970
63-64	1.2	2027	119	5.9	76	3.8	96	4.7	2580	2375
64-65	5	2266	121	5.4	98	4.3	106	4.7	2800	3043
64-65	3.2	2205	139	6.3	93	4.2	99	4.5	2949	2921
64-66	6	4325	232	5.4	164	3.8	190	4.4	5147	5147
65-67	5.2	5010	227	4.5	193	3.8	233	4.7	5484	6013
65-66	3.3	2316	144	6.2	99	4.3	104	4.5	3080	3080
66-67	6.2	~4300							~5000	~5200

Fig. 2. Milk production of test cows on experimental feed. The milk yield was calculated both on the basis of energy (standard milk, 684 kilocalories per kilogram of milk) and of protein (standard milk, 3.2 per cent protein). The large numbers on the curves refer to the test cows, the small numbers to the calving times during the experimental feeding (for example, 3 = Cow No. 3, first calving; 3.2 = Cow No. 3, second calving); the end points of the curves represent the calving days. The annual milk yields may be seen from the curves. Notice the very similar milk production of Cow No. 6 (Metta, 12 years old 1967) during two successive lactation periods.

protein and 4.9 % sugar. The figures in Column 10 show the milk yield calculated on the energy basis; those in Column 11 on the basis of the protein content. Values of 9 kilocalories for fat and 4 kilocalories for protein and sugar per gram were used in the calculations.

The curves of Fig. 2 show how greatly the milk yield has risen since the amount of urea in the feed was increased in 1965. So far, the highest production of standard milk on energy basis per year has been 4217 kilograms; the highest productions for a prolonged lactation period on energy basis have been 5147 kilograms (Cow No. 6, Metta) and 5484 kg (Cow No. 5, Jairu), and on protein basis 6013 kg (Jairu).

The oestrum of the test cows has been regular and easy to observe. However, many of the cows have required several services. The reason for the delay in

fertilization, particularly of the high-yielding test cows is so far unknown.

In any case it is interesting that all of the test cows have become pregnant on the test feed, which excludes all the hundreds or perhaps thousands of known and unknown organic substances which are found in fodder plants.

Studies on the composition of the nitrogenous compounds of the rumen contents have shown that the ammonia concentration in the rumen of cows on normal feed (protein-rich good silage, crushed oat, and hay) two hours after the cows had eaten their ration is higher (11.1 to 26.6 milligrams per 100 ml; average, 15.7 $\pm$ 1.5) than the concentration for test cows (0.7 to 10.6 milligrams per 100 ml; average, 4.8 $\pm$ 1.3). The concentration of ammonia and urea in blood and rumen contents at different times after feeding is shown in Table 2.

The observations on the rapid utilization of ammonia in the rumen of the cows adapted to the test feed could be explained only by supposing that the microbial flora of the rumen content had been effectively adapted to the utilization of ammonium nitrogen. Investigations concerning the microbial flora have shown that the protozoa in the rumen of the adapted cows have disappeared, while the number of bacteria has increased enormously, on average about fifty times (Fig. 3).

In the amino acid composition of the hydrolyzate of the total protein of the rumen contents of test cows and of control cows on normal feed, systematic differences could be found only in respect of α,ε-diaminopimelic acid, a characteristic constituent of the cell wall of many bacteria. It was regularly present in

Table 2. Ammonia- and urea content of blood and rumen contents of test cow Metta

Time of sampling	Daily milk production 8.5 kg st. milk			
	Blood		Rumen contents	
Cow ate in 40 min 2/3 of its ration* 3.6 kg d.m., containing 86 g urea-N + 6 g NH_4-N	NH_3 mg/100 ml	Urea	NH_3 mg/100 ml	Urea
After 0 min first blood sample	0.94	11.50		
After 10 min first rumen sample			32.90	17.48
After 40 min second blood sample	0.69	11.08		
After 80 min third blood sample	1.11	4.36		
After 90 min second rumen sample			4.32	0.44

* The rest of the briquettes were fed only after the sampling was finished.
Quantitative estimations of ammonia and urea were made by Conway's method immediately after sampling.

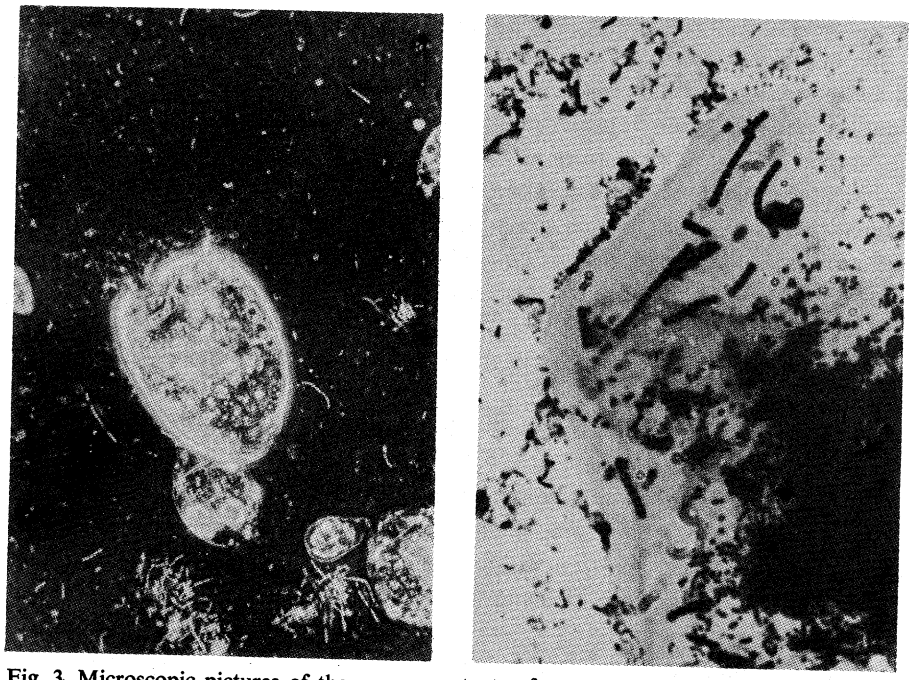


Fig. 3. Microscopic pictures of the rumen contents of cows on normal feed and test feed. Left-hand figure: normal feeding. Plenty of large and small protozoas in addition to bacteria. × 430. Photo M. Lampila.
Right-hand figure: test feeding. Protozoa were not observed microscopically with low magnification. In the picture are seen masses of bacteria of different types. × 2000. Photo T. Ettala.

much higher amounts in the hydrolyzate of the rumen protein of test cows than in that of normally fed cows.

Studies of the composition of the nitrogenous compounds of the faeces suggest that the main part of the nitrogen of the faeces of the test cows is indigestible bacterial protein, containing α,ε-diaminopimelic acid in much higher amounts than in the same fraction from cows on normal feed. This strongly supports the implication of other results that bacterial protein synthesis is greatly enhanced in the rumen of the test cows.

Because the mammary gland receives the raw material for the synthesis of different components of milk from the blood, knowledge of the composition of the blood of the test cows is important. A great number of analyses have been made of the whole blood and plasma of cows on test feed and, for comparison, of cows on normal feed. The blood samples were always taken from the jugular vein.

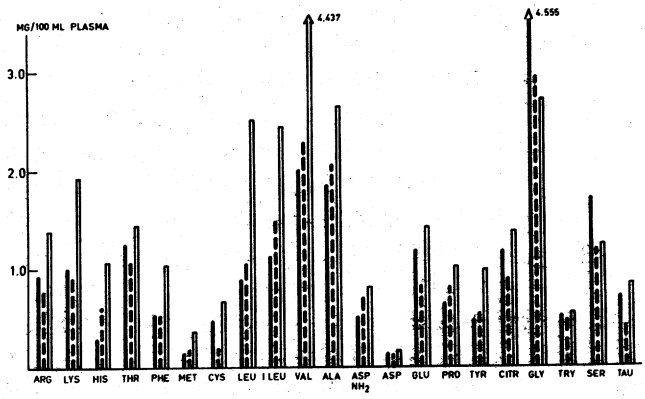


Fig. 4. The amount of free amino acids (mg/100 ml) in the blood plasma of milk-producing cows.
Dark columns: test cows fed on purified protein-free feed.
Dark broken column: cows on feed poor in protein and rich in urea.
White columns: cows on ordinary feed.

The free amino acids of the blood form only a very small part of the nitrogenous compounds of the blood, but they are decisively important for the formation of the proteins of milk in the mammary gland as several authors have shown. The concentration of most of the free amino acids, particularly the essential amino acids, in the blood of the lactating test cows was lower than that in the blood of cows on normal feed. The relative decrease in the concentration of free histidine in plasma was greater in cows on the test feed than the decrease for any other amino acid (Fig. 4).

Low concentrations of free amino acids are found in the plasma of the lactating test cows; the values in the plasma of dry test cows and of heifers are much higher.

The concentration of plasma total nitrogen was similar for the test cows and the control cows whereas the total nitrogen in the blood of the lactating test cows was lower than that for normally fed cows, due to differences in the haemoglobin content (Fig. 5). When the test cows are dry, the haemoglobin

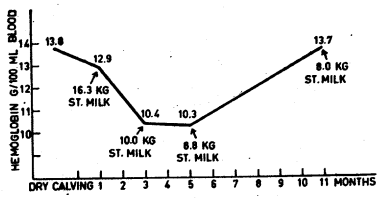


Fig. 5. Hemoglobin content of the blood of test Cow No. 5 (Jairu) 1, 3, 5 and 11 months after calving.

content of their blood is normal, but several weeks after calving the haemoglobin content decreases more than in the blood of normally fed cows. In late lactation, when milk production is low, the haemoglobin content rises gradually; it reaches a maximum when the cows are dry. It is still not known what the reason is for the reduction of the haemoglobin level of a milk-yielding cow on test feed. It may be that the primary reason for this reduction is the deficiency of histidine during the post-calving period, when the protein requirement is greatly increased because of milk production. As is known, the histidine content of haemoglobin is high, more than 8 per cent.

The amino acid composition of the whole-blood protein was usually similar in the test cows and the normally fed control cows. In electrophoretic studies on the serum proteins from several blood samples of the test and control cows, only slight differences in the various protein fractions in samples from individual cows could be observed.

The low amount of ammonium nitrogen in the rumen of the test cows shows the great capacity of the adapted ruminal flora to utilize ammonium nitrogen. This explains why ammonia is not transferred in disturbing amounts to the blood in spite of the large amounts of urea fed.

For the milk-yielding test cows, the total cholesterol and total lipid of blood plasma are shown in Table 3. The higher cholesterol content of 0-milk produced on test feed poor in fat gives support to the view of Patton and McCarthy that cholesterol in its intermediate esterified form probably plays an important role in milk-fat synthesis in the mammary gland. When the oil ration in the feed was increased cholesterol content in 0-milk decreased.

The composition of the 0-milk, when the quantitatively most important compounds are taken into consideration, corresponds to the composition of normal fat-rich milk with respect to fat and protein. The average fat content with the exception of the milk from one test cow, has varied from 4.5 to 6.4 per cent, while the fat content of the milk of the same cows before transfer

Table 3. Total cholesterol and total lipids of blood plasma

	Cholesterol mg/ml	Tot. lipids mg/ml
Test cows	69 ± 3	158 ± 8
Normal cows	210 ± 23	522 ± 64
	Total cholesterol of milk fat (Cholesterol mg/100 g fat)	
Test cows	413 ± 6	
Normal cows	317 ± 4	

to the test feed ranged from 4.2 to 4.8 per cent. The protein content of 0-milk has been exceptionally high, 3.8 to 4.3 %. When the content of urea in the feed was raised the fat content remained about the same but the protein content rose remarkably. It thus seems that the amount of urea fed has a positive influence on the protein content of the test milk but not on the fat content. The sugar content of the 0-milk, 4.4 to 4.7 per cent, was lower than that of normal milk.

The composition of the nitrogenous substances, particularly the proteins, of the 0-milk has received our special attention. The amino acid composition of total protein and casein has been estimated, after acid hydrolysis, in hundreds of milk samples. The values for the amino acids of the total protein and casein of the test milk are so close to those of milk produced on normal feed that no conclusive differences could be demonstrated. Casein, non-protein-N, ammonium- and urea-N of 0- and normal milk are shown in Table 4.

Table 4. Composition of total-N compounds of milk

	Casein % of total crude protein	Non-prot.-N % of total-N	NH_4-N (% of total-N)	Urea-N (% of total-N)
0-milk	77.5 ± 0.60	3.95 ± 0.13	0.30 ± 0.02	0.82 ± 0.11
Normal milk	78.5 ± 0.51	4.2 ± 0.35	0.23 ± 0.02	1.71 ± 0.08

Nor has the fractionation of the proteins shown any differences between the test milk and normal milk. Both the direct fractionation of the proteins of milk on a DEAE-cellulose column and the fractionation of casein and the whey proteins of milk by means of gel electrophoresis have shown the similarity between the 0-milk and normal milk. Small differences in some smaller protein fractions of the milk of different cows have been observed, but they seem to be of an individual character. No conclusive differences between 0-milk and normal milk could be found in the activities of the milk enzymes studied when allowance had been made for the appreciable individual variations. So far, peroxidase, xanthineoxidase, aldolase, amylase, alkaline phosphatase, and lipase activities have been determined.

The results show how extraordinarily effective the protein synthesis in the mammary gland is. In spite of the low concentration of some free essential amino acids in the blood of the test cows, the mammary gland is capable of synthesizing a surprising amount of milk protein, the numerous components of which have a normal composition. The high protein content and low urea and ammonium content of 0-milk are particularly remarkable.

Table 5. The fatty acid composition of the milk fat on test feed and normal feed (E. Piironen)

Date of calving	Eiru 11.12.1963	Jairu 24.12.63	Jairu 19.5.65	Normal cattle
Feeding period				
began	1.4.64	11.1.64	11.7.65	25.9.63
ended	30.6.64	20.2.64	20.9.65	15.5.64
lasted days	91	41	72	234
Fat in diet g/day				
Olive oil	36.6	0.0	36.6	
Maize oil	18.4	18.4	46.0	
Linseed oil	18.6	18.6	46.5	
Total amount	73.6	37.0	129.1	
Production of milk kg/day	6.09	7.61	10.65	
Production of fat kg/day	0.342	0.358	0.559	
Fat %	5.61	4.71	5.24	
	Fatty acid % of total fatty acid			
Samples	6	3	6	8
Fatty acids:				
4 : 0	2.58	2.63	3.05	3.05
5 : 0	0.12	0.13	0.05	< 0.02
6 : 0	1.88	1.90	1.75	1.76
7 : 0	0.14	0.12	0.05	< 0.02
8 : 0	1.30	1.08	1.12	1.02
9 : 0	0.18	0.12	0.06	< 0.02
10 : 0	3.63	2.38	2.73	2.13
10 : 1	0.54	0.44	0.40	0.25
11 : 0	0.30	0.22	0.14	< 0.02
12 : 0	5.41	3.64	3.74	2.65
12 : 1	0.20	0.19	0.18	0.08
13 : obr	0.20	0.15	0.17	0.03
13 : 0	0.55	0.51	0.26	0.04
14 : obr	0.18	0.32	0.21	0.09
14 : 0	11.99	10.17	10.95	9.87
14 : 1	1.63	2.63	1.95	1.01
15 : obr	0.80	0.52	0.82	0.50
15 : 0	4.09	4.88	2.70	0.90
16 : obr	0.11	0.48	0.33	0.19
16 : 0	40.29	48.16	38.90	28.41
16 : 1	3.54	4.29	4.22	2.03
17 : obr	1.08	0.94	0.96	0.58
17 : 0	1.21	1.57	0.90	0.46
17 : 1	0.77	1.13	0.77	0.39
18 : 0	1.93	0.98	2.58	12.29
18 : 1	13.18	8.35	19.14	29.37
18 : 2	1.48	1.40	1.15	1.79
18 : 3	0.68	0.67	0.74	1.11

In a comparison of test milk and normal milk, the only major constituent showing large differences in composition is the fat. As is well known, the composition of milk fat is greatly influenced by the nature and amount of the fatty acids in the feed of the cow.

If the amount of the vegetable oil fed to the test cows is very low, *e.g.* 37 g per day, the palmitic acid content of the fat of the 0-milk is high, nearly 50 %, and the oleic acid content low, only 8 % of the total fatty acids. The content of stearic acid is only 1 % (Table 5). This shows that only a very small amount of fatty acids with 18 or more carbon atoms is synthesized in the mammary gland. If greater amounts of oil are fed, the amount of palmitic acid decreases markedly, the amount of oleic acid increasing correspondingly. These results suggest that the fatty acids higher than palmitic acid are mainly transferred to the mammary gland from the blood. Considering the high fat content of the 0-milk and the very low lipid content of the blood of the test cows, an unusually vigorous synthesis of fatty acids and fat must take place in the mammary gland from acetic acid, but probably also from glucose. The low sugar content of the 0-milk would thus be explained. It is noteworthy that studies of the rumen content of test cows have shown that the ratio of propionic acid to acetic acid is exceptionally high, and in spite of that the 0-milk is rich in fat and poor in sugar.

The vitamin content of the 0-milk has been followed from 1963 to 1965. To judge by the determinations of the water soluble vitamins, the biosynthesis of the vitamin-B complex brought about by the microorganisms in the rumen seems to be rapid enough to maintain a normal content of these vitamins in the test milk (Table 6).

Regarding the fat-soluble vitamins, their concentration in milk depends on

Table 6. Vitamin content of milk from test cows and from normally fed cows during the years 1964 and 1965. (After M. Saarivirta)

Thiamin HCl (μg/ 100 ml)	Ribo-flavin (μg/ 100 ml)	Nicotinic acid (μg/ 100 ml)	Pyri-doxine (μg/ 100 ml)	Folic acid* (μg/ 100 ml)	Biotin (μg/ 100 ml)	Calcium panto-thenate (μg/ 100 ml)	B_{12} (mμg/ 100 ml)	Ascorbic acid (mg/ 100 ml)
Milk from test cows								
45.7±1.8	306±18	167±4.9	57.8±5.2	2.91±0.15	3.42±0.31	1120±55	455±46	2.78±0.12
Mixed milk from normally fed cows								
43.4±2.6	293±32	158±8.4	53.0±6.3	3.18±0.19	3.13±0.84	597±62	523±48	2.25±0.13

* Values from 1963.

the amount fed. A daily dose of 400 mg DL-α-tocopherol during a year did not have any demonstrable effect on the fertility or milk production. On the other hand the calves seem unable to grow up into milking cows on the protein-free test feed without a vitamin-E supplement.

The flavour of the 0-milk has aroused particular attention in our laboratory. In organoleptic tests the 0-milk was found to have a characteristic milk flavour without any off-flavours. Thus it appears that the basic flavour substances of milk are formed in the rumen and other organs of the cow.

It is not possible here to deal with the problem of milk flavour in greater detail. I will mention only that δ-lactones — probably the most important flavour substances of milk — occur in test milk, on the average, at least in the same concentrations as in normal milk. The concentrations of δ-lactones from C_6 to C_{10} are similar in 0-milk and normal milk, whereas the concentration of C_{12} and C_{14} lactone is higher in 0-milk. Gas-chromatographic analysis and mass-spectrometric identification of δ-lactones and other flavour compounds in milk have been used in our laboratory.

Fodder plants contain a great number of aliphatic aldehydes, ketones, alcohols and esters. By means of a plastic tube, pure compounds of these groups were passed into the rumen of a lactating cow on purified test feed. Most of the substances tested were transferred in trace amounts to the milk, the maximum concentration being reached, in general, after 2 hours. Very few flavour substances are present in the fodder plants in such amounts that they could occur in milk in concentrations which would exceed the flavour threshold. This explains why milk flavour is not distinctly influenced by feed which does not have a pronounced off-flavour. It is true that some recent observations on the synergistic effects of different flavour compounds in sub-threshold amounts reduce the supposed usefulness of gas-chromatographic analysis of milk flavour. It is therefore all the more important that the 0-milk has made it possible to compare organoleptically normal milk produced on different types of feed with milk produced on purified diet.

As the feeding experiments with purified nutrients had shown decisively that the cow is able to use urea- and ammonium nitrogen for protein synthesis to such an extent that it is adequate both for maintenance and for an annual milk yield of 4000 kg, it seems probable that urea can be used successfully in large amounts as a substitute for protein in normal feed poor in protein, in other words that the protein problem in milk production at a fairly high level could practically disappear. Feeding experiments with different fodder combinations low in protein, which are in progress with six test cows, give support to this conclusion. We have tried to arrange the rations so that the digestible true protein in the feed of different cows should be on different levels.

Table 7. The annual amounts of feed consumed by Lila, and the annual milk yield

Lila born Sept. 14, 1963, calved the first time Feb. 26, 1966								
Fed	kg	fu	Total N kg	Dig. raw prot. N kg	True prot. N kg	Dig. true prot. N kg	Dig. urea N kg	Dig. 'amide' N kg
Potatoes (fresh weight)	6160	1176	15.4	13.8	6.7	6.1		7.7
Sugar beet pulp, dry	1171	900	19.8	9.8	13.8	7.9		1.9
Oat straw	561	175	2.5	1.0	2.3	0.7		0.3
0-fiber	578	193						
Hemicellulose powder	847	770						
Urea	136		63.5				44.4	
Fat from the fodders fed	35							
Fat from the veget. oils	34	55						
Mineral mixture	212							
Vitamin A	36.4	mill. I. U.						
Vitamin $D_2 + D_3$	7.3	mill. I. U.						
Vitamin E	202 g							
Total		3076	101.2	24.6	22.8	14.7	44.4	9.9
Milk yield:								
Milk produced			4777 kg					
Milk calculated on an energy basis			4873 kg					
Milk calculated on a protein basis			4825 kg					
Milk produced per year contained:								
Dry matter	636 kg		13.3 %					
Fat	198 kg		4.14 %					
Protein	155 kg		3.24 %					
Sugar	234 kg		4.89 %					

The first animal, Lila, was a heifer which was adapted during 40 days before calving to the urea-rich feed as low in digestible true protein as possible in Finnish conditions. Her consumption of feed and her milk yield per year are seen in Table 7.

On this feed the cow produced 4777 kg milk per year with 4.1 % fat, corresponding to 4873 kg standard milk. The composition of milk produced on this feed has been almost the same as that of standard milk and not exceptionally rich in fat and protein as is the 0-milk. Only about 20 % of the digestible total nitrogen of the feed used by this cow has been digestible true protein nitrogen, the main part being urea-N (Table 8). Lila will calve for the second time in August and has produced 6003 kg standard milk during her first, prolonged lactation period (Fig. 6). Lila's milk production is considerably higher than that of Metta, the best test cow on the purified feed. It is possible but not demonstrated that the digestible true protein in the feed, 250 g/day, brings about the rise in the milk production.

Table 8. The amounts of digestible raw protein, digestible true protein and 'amide' fraction in different fodders consumed by cow Lila in one year

	Dig. raw prot. kg	Dig. true prot. kg	Dig. raw prot. from urea kg	Dig. 'amide'-fraction
Potatoes	86	38		48
Supar beet pulp	61	49		12
Oat straw	6	4		2
Urea	278		278	
Total	431	91	278	62
% from dig. raw prot.		21.1	64.5	14.4

Five more cows were included in experiments of a practical nature in the autumn of 1966. One of them, Kelo, had calved twice during normal feeding. Adaptation to the urea feeding took place during 40 days. The composition of the daily ration of Kelo during 7 months is seen in Table 9. The annual milk yield will probably reach 5400 kg of standard milk (Fig. 7). About 30 % of the digestible crude protein-N in the feed of Kelo is digestible true protein-N. Kelo's younger sister Lelo who calved later, may reach about the same annual milk yield on similar feed. Both cows are of a relatively small size, the normal weight being about 430 kg. The concentration of the milk of Kelo corresponds very closely to that of standard milk. The contents of free amino acids in the

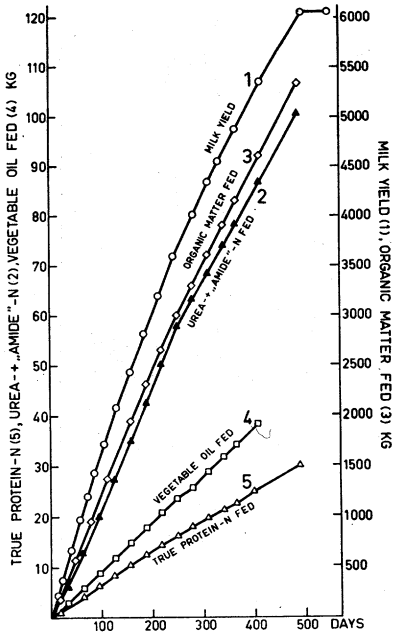


Fig. 6. Lila's milk production and consumption of different feedstuffs (s. Table 7).

blood plasma of these cows, on feed poor in protein and rich in urea, are seen in Fig. 4.

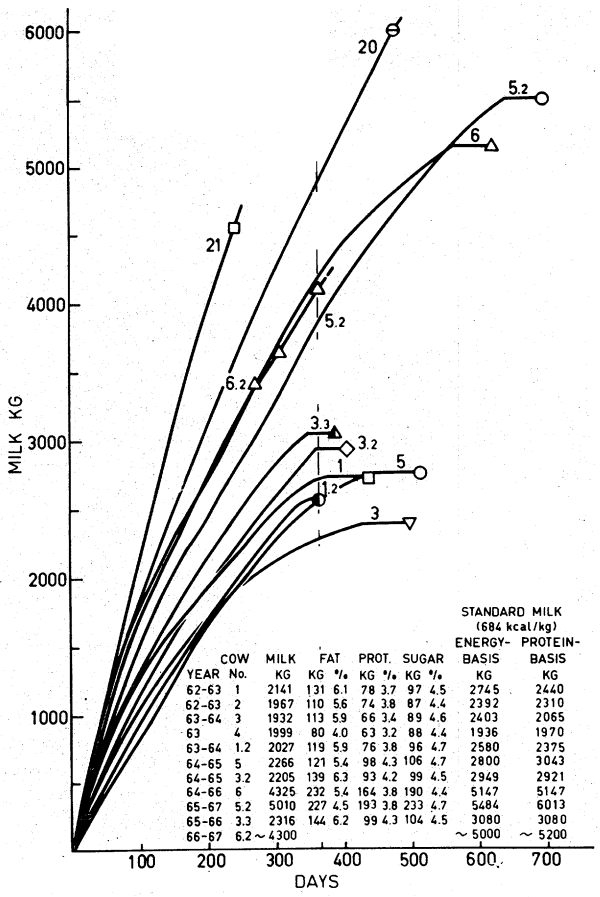
Our experiments have opened new views for cattle feeding and milk production. The experiments performed with purified protein-free nutrients have laid the basis for a new development, although natural feed without any protein does not exist. As it is known approximately how large a milk production can be reached with urea and ammonium salts as the sole sources of nitrogen, various practical feed combinations can be planned, as I have reported above. On the basis of the available scanty material one could draw the conclusion that if the cow produces 4000 kg milk on a protein-free feed with urea as the sole source of nitrogen, the addition of about 20 % of digestible true protein in common feed stuffs (urea being the main source of nitrogen) will raise the milk yield to about 5000 kg per year, and the addition of 30 % of digestible true protein will raise it to about 5500 kg.

This rough calculation holds true only in the case where protein is the only limiting factor in the protein-free feed used in our experiments. It is, however, quite possible that other factors not present in the purified feed would have a favourable effect on the microbial flora in the rumen, or otherwise promote milk

Table 9. Composition of the daily ration of cow Kelo during 7 months after calving (expressed as dry matter)

Urea	Cats	Barley	Sugar beet pulp	Hemi-cellulose	0-fiber	Oat straw	Salt mixture	Vitamin A	Vitamin D
g	kg	kg	kg	kg	kg	kg	g	IU	IU
440	4.7	1.3	3.9	2.9	0.5	0.9	300 + 70 $CaCO_3$ + 50 NaCl + 24 Na_2SO_4	100 000	20 000

Milk yield during 7 months 4135 kg standard milk



YEAR	COW No.	MILK KG	FAT KG	FAT %	PROT. KG	PROT. %	SUGAR KG	SUGAR %	STANDARD MILK (684 kcal/kg) ENERGY-BASIS KG	STANDARD MILK (684 kcal/kg) PROTEIN-BASIS KG
62-63	1	2141	131	6.1	78	3.7	97	4.5	2745	2440
62-63	2	1967	110	5.6	74	3.8	87	4.4	2392	2310
63-64	3	1932	113	5.9	66	3.4	89	4.6	2403	2065
63	4	1999	80	4.0	63	3.2	88	4.4	1936	1970
63-64	1.2	2027	119	5.9	76	3.8	96	4.7	2580	2375
64-65	5	2266	121	5.4	98	4.3	106	4.7	2800	3043
64-65	3.2	2205	139	6.3	93	4.2	99	4.5	2949	2921
64-66	6	4325	232	5.4	164	3.8	190	4.4	5147	5147
65-67	5.2	5010	227	4.5	193	3.8	233	4.7	5484	6013
65-66	3.3	2316	144	6.2	99	4.3	104	4.5	3080	3080
66-67	6.2	~ 4300							~ 5000	~ 5200

Fig. 7. Milk production of test cows on purified, protein-free feed (1-6), of Lila (20) and of Kelo (21). Details in the text.

production. I hope that an experiment using purified feed with urea and purified protein as the nitrogen sources, started in May, will throw light on this important problem. In figures 8-10 photographs of some of the experimental cows are presented.

As the surprisingly vigorous bacterial protein synthesis in the rumen from urea and ammonium nitrogen has been demonstrated, protein-free material which has not been used previously in the feeding of cows can be included in the feed. In countries rich in forests, the hemicellulose found in large amounts in wood is a product which according to our experiments can replace about 30 per cent of the total feed of cows when a sufficient amount of urea is used.

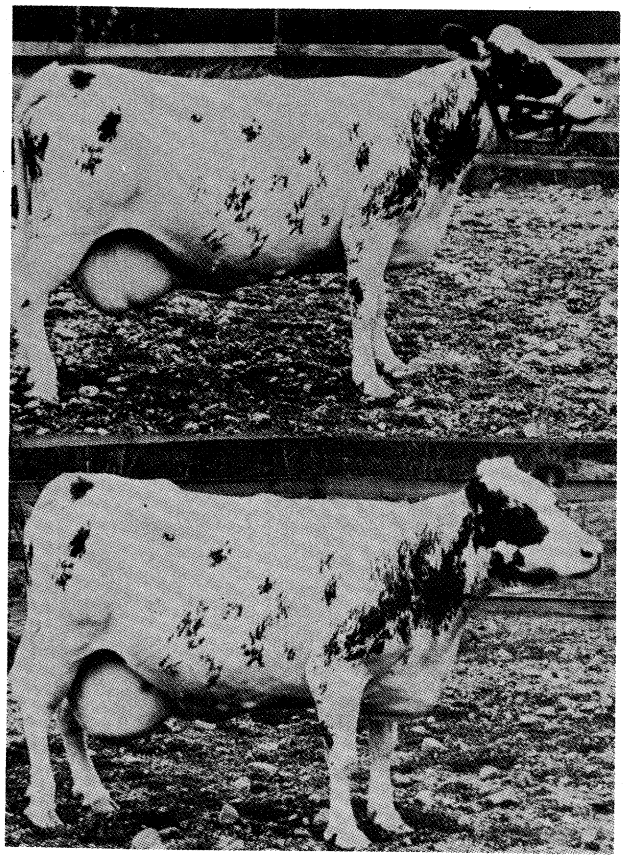


Fig. 8. Test cow Metta (No. 6) after being on the purified, protein-free test feed for 1 year (at the top) and for 3 years (below).

Hemicellulose can be hydrolyzed and separated from wood by means of superheated steam. The so-called 0-fibre, which in addition to short cellulose fibres also contains other substances, *e.g.* plenty of fat, is a waste product which together with hemicellulose in many countries pollutes the lakes and rivers.

In tropical areas surgar cane, which gives greater yields than any other cultivated plant, offers plenty of suitable feed for cattle. Plants rich in starch, such as sweet potato and manioc also grow well in a tropical climate. The date palm also can provide feed for cows. The lack of protein has up to now prevented the use of sugar- and starch-rich crops for feed. In the future it is possible that chemical industry will be able to produce sufficiently cheap products such as organic acids, glycerol and other similar compounds, which at

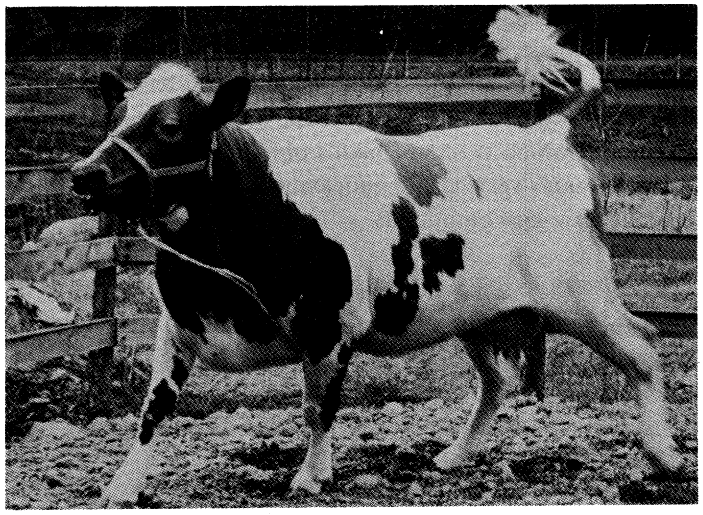


Fig. 9. Heifer Lila after being on a feed poor in protein and rich in urea for 16 months and producing 6000 kg st. milk.

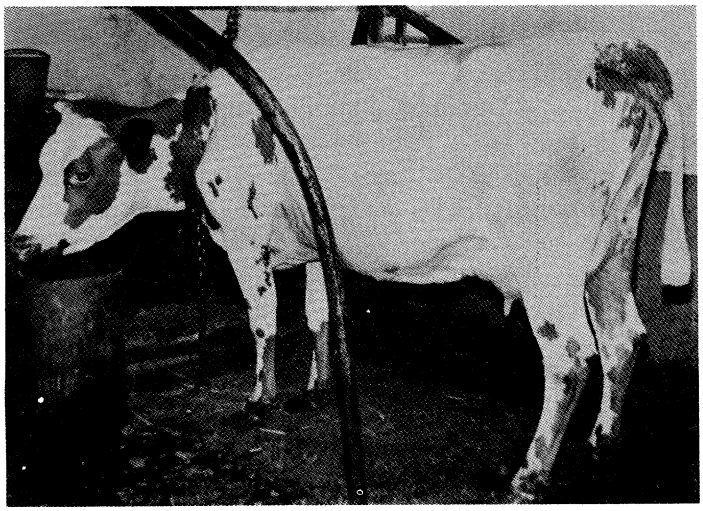


Fig. 10. Cow Kelo, 8 months on a feed poor in protein and rich in urea.

least in a smaller proportion are suitable for use as feed. The idea which as proposed previously concerning the extraction of proteins from grass and the use of the resulting protein preparation as the food of man, seems now theoretically possible as an extreme measure, as the protein removed can be replaced

in the extracted grass by urea, to provide a feed suitable for cattle. A large proportion of the protein is easily extracted from the grass into solution. When nowadays new ways are sought for the production of protein, *e.g.* the cultivation of suitable yeasts using petroleum or methane as substrates, it is important to know that the cow, which is one of man's oldest domestic animals, is able to comply even with man's very newest endeavours to produce high-value proteins using only simple nitrogen compounds.

LITERATURE

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